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NITROGEN RETENTION ACROSS A GRADIENT OF ^{15}N ADDITIONS TO AN UNPOLLUTED TEMPERATE FOREST SOIL IN CHILE

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Abstract. Accelerated nitrogen (N) inputs can drive nonlinear changes in N cycling, retention, and loss in forest ecosystems. Nitrogen processing in soils is critical to understanding these changes, since soils typically are the largest N sink in forests. To elucidate soil mechanisms that underlie shifts in N cycling across a wide gradient of N supply, we added $^{15}NH_4{}^{15}NO_3$ at nine treatment levels ranging in geometric sequence from 0.2 kg to 640 kg $N \cdot ha^{-1} \cdot yr^{-1}$ to an unpolluted old-growth temperate forest in southern Chile. We recovered roughly half of ^{15}N tracers in 0–25 cm of soil, primarily in the surface 10 cm. Low to moderate rates of N supply failed to stimulate N leaching, which suggests that most unrecovered ^{15}N was transferred from soils to unmeasured sinks above ground. However, soil solution losses of nitrate increased sharply at inputs >160 kg $N \cdot ha^{-1} \cdot yr^{-1}$, corresponding to a threshold of elevated soil N availability and declining ^{15}N retention in soil. Soil organic matter (<5.6 mm) dominated tracer retention at low rates of N input, but coarse roots and particulate organic matter became increasingly important at higher N supply. Coarse roots and particulate organic matter together accounted for 38% of recovered ^{15}N in soils at the highest N inputs and may explain a substantial fraction of the ''missing N'' often reported in studies of fates of N inputs to forests.

Contrary to expectations, N additions did not stimulate gross N cycling, potential nitrification, or ammonium oxidizer populations. Our results indicate that the nonlinearity in N retention and loss resulted directly from excessive N supply relative to sinks, independent of plant–soil–microbial feedbacks. However, N additions did induce a sharp decrease in microbial biomass C:N that is predicted by N saturation theory, and which could increase long-term N storage in soil organic matter by lowering the critical C:N ratio for net N mineralization. All measured sinks accumulated ^{15}N tracers across the full gradient of N supply, suggesting that short-term nonlinearity in N retention resulted from saturation of uptake kinetics, not uptake capacity, in plant, soil, and microbial pools.

Key words: ammonium nitrate; dissolved organic nitrogen; nitrification; nitrogen fertilizer; nitrogen saturation; ^{15}N stable isotope; nutrient budget; old-growth temperate forest; roots; soil organic matter.

INTRODUCTION

Nitrogen supply often limits plant growth in temperate forests, a pattern that is attributable to the scarcity of N in bedrock, low inputs from atmospheric deposition, constraints on N fixation, and the persistence of N losses that escape direct biotic control (Vitousek and Howarth 1991, Hedin et al. 1995, Vitousek et al. 1998). One consequence of low N supply is strong retention and efficient recycling of available N. Soil subsystems dominate these N cycling processes, where plants, microbes, and abiotic processes together compete for and effectively limit available N losses to levels as low as <1% of actively cycling pools (Perakis and Hedin 2001). Transfers of N from fast to slow turnover pools, as from soil microorganisms (e.g., mycorrhizae and rhizosphere bacteria) to plants and eventually soil organic matter, can promote even longer term N retention (Johnson 1992, Currie et al. 1999). Ironically, these transfers ultimately may reinforce N limitation by sequestering ~90% of ecosystem N capital in slow turnover pools of recalcitrant soil organic N (Cole and Rapp 1981), with potential for subsequent loss from soils and ecosystems as dissolved organic N (Perakis and Hedin 2002).

Most unpolluted temperate forests receive low background inputs of N (<5 kg $N \cdot ha^{-1} \cdot yr^{-1}$) from natural atmospheric deposition and asymbiotic N fixation (Holland et al. 1999, Cleveland et al. 1999). Symbiotic N fixation, air pollution, and forest fertilization can however supply substantially more N over time scales that range from days, to decades, and to ''chronic'' conditions. Symbiotic N fixation in several ecologically important tree genera (e.g., *Alnus*, *Myrica*, *Robinia*) can add up to 50–200 kg $N \cdot ha^{-1} \cdot yr^{-1}$, which enriches soils

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with many thousands of kilograms of nitrogen per hectare over the period of stand development (Binkley et al. 1992, Cleveland et al. 1999). Chronic deposition of atmospheric N pollutants near industrialized regions can deliver N inputs that range from a doubling of background levels to >80 kg $N \cdot ha^{-1} \cdot yr^{-1}$ (Holland et al. 1999, Lilleskov et al. 2002). Deliberate N fertilization to increase wood growth and fiber production is also common, with N typically added in a single large dose of up to 900 kg/ha, to minimize cost and increase tree N uptake over competitive sinks in soils (Miller et al. 1976). In all cases, the onset of significant nitrate (NO_3^-) production and leaching represents a critical switch in the cycling of available N from N-retentive to N-leaky conditions that may also cause undesirable changes in availability of other essential (e.g., Ca, Mg, K) and toxic (e.g., Al) elements (Aber et al. 1998).

Nonlinear shifts in the forest N cycle during the transition from N-limited to N-saturated conditions are a key yet understudied component of the ''nitrogen saturation'' hypothesis. Shifts in soil N processing may be especially important, where changes in either the production or consumption of N can drive nonlinear shifts in N retention at the ecosystem level. Autotrophic nitrification, the conversion of ammonium (NH_4^+) to NO_3^- by soil bacteria, is considered a pivotal process in this shift (Aber et al. 1998). At low N supply, intense competition for available N in the plant–soil–microbe system can limit NH_4^+ availability and population sizes of ammonium oxidizing bacteria (Zak et al. 1990). Enhanced N availability may, however, relax competition for N, thus stimulating ammonium oxidizers, and leading to elevated production and hydrologic loss of NO_3^-. Yet despite widespread evidence that added N can drive nonlinear increases in net NO_3^- production and loss from soils, it remains unclear to what degree these changes result from stimulation of ammonium oxidizing bacteria, or from the saturation of NO_3^- uptake by plants, free-living heterotrophic microbes, mycorrhizae, and abiotic reactions.

We here report on an experiment in which we use ^{15}N to trace the cycling and fate of N additions to an unpolluted temperate forest soil across a broad range of input rates. Our interest was in understanding the mechanisms responsible for retention of added N, and in examining whether N production and consumption responded nonlinearly to increasing rates of N addition. We adopted a novel N addition strategy that depended on enriching soils with ^{15}N across a geometric increase in N inputs within a single site, and documenting the response by each of the major mechanisms: loss, plant uptake, soil uptake, and microbial uptake. This approach differs from traditional fertilization experiments that examine responses to only several rates of N addition (e.g., control, low, high) by elucidating the dose–response response pattern of N saturation to a wide range of N inputs, as well as using ^{15}N tracers to identify mechanisms responsible for shifts in N retention and loss.

Because it is often difficult to separate the effects of added N from other human disturbances when investigating changes in N cycling, we added N to an old-growth temperate forest of southern Chile with no significant history of air pollution or other disturbances. We focused on soil N cycling for both practical and strategic reasons; high structural complexity in old-growth forests requires very large plots with prohibitive costs to effectively track ^{15}N into aboveground biomass, and previous studies of aggrading forests, where plant sinks should be strongest, have nevertheless identified soils as the dominant sink for ecosystem level ^{15}N additions (e.g., Nadelhoffer et al. 1999). We hypothesized that N additions would drive a nonlinear pattern of N retention and NO_3^- loss, with changes in the nitrification step of the N cycle defining the shift from N-retentive to N-leaky conditions.

Methods

Site description

We conducted this research in coastal montane old-growth forest of Parque Nacional Chiloé, Chile (42°30′ S, 74°03′ W, 550 m above sea level). The climate is temperate wet maritime, with mean temperature of 4.2°C in winter (July–September), 10.2°C in summer (January–March), and 5500 mm annual precipitation. Marine aerosols dominate atmospheric inputs and are an important source of nutrients to plants, soils, and stream waters (Hedin et al. 1995, Kennedy et al. 2002). Wet deposition of inorganic N is among the lowest globally (~1.3 kg $N \cdot ha^{-1} \cdot yr^{-1}$) with additional N supplied by fog (~0.5–1.5 kg $N \cdot ha^{-1} \cdot yr^{-1}$; K. Weathers, W. Keene, J. Moody, T. Walter, M. Brown, G. Lovett, J. Armesto, J. Galloway, G. Likens, C. Perez, A. Johnson, and L. Hedin, *unpublished manuscript*), and asymbiotic fixation (1.5–3.5 kg $N \cdot ha^{-1} \cdot yr^{-1}$, Perez et al. 2003). Whole ecosystem nutrient capital is comparable to other montane temperate forests (Vann et al. 2002).

We studied mixed evergreen angiosperm–gymnosperm forests representative of large areas of southern Chile (Armesto et al. 1995). These forests display a gap-phase disturbance regime, and support a mixture of early-successional and late-successional species dominated by *Nothofagus* spp. Understory and herbaceous layers are patchy, and the bamboo *Chusquea quila* is common. Coarse woody debris is abundant (Carmona et al. 2002).

Soils are developed from highly weathered schists (Watters and Fleming 1972) and range in depth from 40 cm on ridges to >100 cm on slopes (Table 1). A thin litter horizon (C:N [by mass] = 62) turns over rapidly to form organic-rich surface soils (0–10 cm: 23% C, C:N = 30; Perez et al. 1991) with high fine root biomass (2.9 Mg/ha fine roots <2 mm diameter, 0–10 cm). Subsurface soil (10–25 cm, 8% C, C:N =

TABLE 1. Summary of ^{15}N treatments and selected plot characteristics prior to start of nitrogen additions.

N added (kg/ha)	^{15}N enrichment (%)†	Bulk density (g/cm^3)‡		C:N ratio (by mass)			
					Soil		
		0–10 cm	10–25 cm	Litter	0–10 cm	10–25 cm	Fine root (0–10 cm)
0.2	99	0.09	0.26	57	30	24	47
5	20	0.21	0.54	67	25	17	54
10	20	0.25	0.49	52	31	19	51
20	10	0.21	0.54	72	30	19	51
40	10	0.08	0.46	66	35	20	64
80	5	0.14	0.41	62	31	21	51
160	5	0.18	0.67	62	33	17	61
320	2.5	0.36	0.67	58	24	16	60
640	2.5	0.09	0.18	59	32	23	52

† Enrichment of ^{15}N additions is presented as atom percentage excess over natural abundance.
‡ Bulk density is calculated on the <5.6 mm fraction; $n = 6$ samples.

20) is clay rich, with fewer fine roots and poor hydraulic conductivity (Salmon et al. 2001, Vann et al. 2002).

Plot installation and nitrogen additions

We established nine 12-m^2 plots in December 1997 in a typical area of steep (60–70%) slopes, and avoided locations of recent disturbance and exposed mineral soil. Each plot was randomly assigned an N addition treatment from the geometric range 0.2, 5, 10, 20, 40, 80, 160, 320, and 640 kg N·ha^{-1}·yr^{-1}. We added N as $^{15}NH_4{}^{15}NO_3$ at enrichments ranging from 2.5% to 99 atom percentage excess ^{15}N (Table 1) to add roughly the same amount of ^{15}N atoms in each treatment. Throughout, we refer to ^{15}N quantities (e.g., ^{15}N added, ^{15}N recovery, ^{15}N distribution) as representative of this range of N additions. The ^{15}N additions began in December 1997 (austral summer) and continued for one year, with the total annual N input divided into equal monthly doses. Monthly N additions were dissolved in deionized water equivalent to a 1-mm precipitation event, and were dispersed evenly across the plot using >20 passes with a backpack sprayer.

Soil solution sampling and analysis

Two quartz lysimeters (0.2 μm pore size, Prenart Corporation, Copenhagen, Denmark) were used to sample soil water at 10-cm and 40-cm depths in each plot. Lysimeters were installed by removing a 2.2 cm diameter core of soil to within 5 cm of the final depth, gently pressing the lysimeter into full contact with soil, and backfilling the access hole. Installation occurred two weeks (10 and 640 kg N/ha), nine months (0.2, 5, and 160 kg N/ha), or two years (20, 40, 80, and 320 kg N/ha) prior to the start of N additions.

We sampled lysimeters 2–3 wk after each monthly N addition by applying 30 cm Hg vacuum for 48 h to draw soil water into sealed dark 1.5-L polyethylene bottles. Two replicate 60-mL samples were removed by syringe, filtered immediately through pre-rinsed Gelman A/E glass fiber filters into clean polyethylene bottles, and one replicate was preserved with 0.2 mL chloroform. Samples were transported cold via express mail to Cornell University and analyzed for dissolved N species (Hedin et al. 1995). Ammonium (NH_4^+) was measured by Alpkem automated colorimetry (OI Analytical, College Station, Texas, USA). Nitrate (NO_3^-) was measured by Dionex ion chromatography (Dionex, Marlton, New Jersey, USA). Total dissolved N was determined by high temperature persulfate digestion, followed by analysis as NO_3^- using Alpkem colorimetry.

Soil sampling and analysis

We sampled soils twice to estimate N contents and ^{15}N enrichments, one week prior to the start of N additions to establish background values, and three weeks after the cessation of N additions to estimate ^{15}N recovery. Three random locations in each plot were sampled for litter, surface soil (0–10 cm), and subsoil (10–25 cm). Litter (Oi and Oe horizons) was sampled with a 15 × 15 cm template. Surface soil was excavated in the same 15 × 15 cm area using a 10 cm deep pit. At the base of the 0–10 cm pit, we sampled subsoil from 10–25 cm using a 5.4 cm diameter corer.

Samples were processed in the field immediately after collection. Surface and subsoil samples were weighed and wet sieved at 5.6 mm to facilitate rapid field processing. Sieved subsamples (200 g) were set aside for moisture, total C and N, and ^{15}N determination. Gravimetric soil moisture was determined after drying at 105°C. Organic matter retained on the sieve was separated into fine roots (<2 mm), coarse roots (2–10 mm), and particulate organic matter (POM) >5.6 mm. Fine roots were rinsed vigorously in deionized water to remove adhering soil. Coarse roots and POM were scrubbed with a stiff brush and rinsed with water to remove soil. Litter, organic matter, and root samples were dried at 65°C and ground to powder for C, N, and ^{15}N analysis by Europa Scientific ANCA-2020 (PDZ Europa, Cheshire, UK) at the Utah State University Stable Isotope Facility.

A weighted composite of sieved 0–10 cm soils from each plot was processed further to examine N pools in greater detail. Live roots that had passed through the

sieve were removed from a 100-g composite sample using tweezers, cleaned, and set aside for analysis. Available NH_4^+ and NO_3^- were extracted from 40 g of root-free soil for 1 h with 100 mL of 2 mol/L KCl, filtered with rinsed Whatman #1 (11 μm nominal pore size), and analyzed using Alpkem automated colorimetry. Total dissolved N was extracted from 13 g of root-free soil for 1 h with 35 mL 0.5 mol/L K_2SO_4, filtered, and analyzed as NO_3^- following high temperature persulfate digestion (Perakis and Hedin 2001).

Nitrogen transformations were measured in a composite subsample of 0–10-cm root-free soil from each plot at the end of N additions. Gross N production was measured by ^{15}N pool dilution. Solutions of 99 atom percent $^{15}NH_4Cl$ (15 μg N/g dry soil) or $Na^{15}NO_3^-$ (5 μg N/g) were mixed with sieved field-moist soil, and subsamples were extracted with KCl after 5 min and 12 h for ^{15}N. Samples receiving $^{15}NO_3^-$ were incubated with and without acetylene to separate heterotrophic and autotrophic nitrification (Hart et al. 1997). Net N production (NH_4^+ and NO_3^-) was estimated by field incubation of 65 g of sieved field-moist soil in buried polyethylene bags for 10 d and 28 d.

Soil microbial C and N were assayed using a liquid chloroform direct-extraction technique developed for these soils (Perakis and Hedin 2001); 1.5 mL $CHCl_3$ was added directly to 13 g root-free soil, sealed airtight for 2 d, extracted with 35 mL 0.5 mol/L K_2SO_4, and filtered prior to analysis for dissolved organic C using a Shimadzu TOC 5000A analyzer (Shimadzu, Tokyo, Japan) and total N by persulfate digest. Potential nitrification was assayed in the field laboratory by combining 2 g fresh soil with 50 mL ammonium phosphate solution, and measuring NO_3^- immediately and after 12 d of incubation. We also determined autotrophic ammonium oxidizers by most probable numbers culture (Schmidt and Belser 1994) using both full strength and 1/10th strength ammonium stock solutions in the serial dilutions, since some nitrifiers grow better at low ammonium concentrations (Donaldson and Henderson 1989). Microbial respiration was measured by incubating 3 g fresh soil with roots removed in sealed jars, and measuring CO_2 production before and after 24 h using a LICOR 6200 (LICOR, Lincoln, Nebraska, USA).

Calculations and statistics

Dissolved organic nitrogen (DON) in soil water and extracts was calculated as total dissolved N minus NH_4^+ minus NO_3^-. Microbial C and N were calculated as the difference of dissolved organic C and total N between chloroform treated and untreated samples. Nitrogen in soil organic matter (SOM) was calculated as the total N of <5.6 mm soil minus root N minus microbial N minus total soluble N. Total N of organic matter >5.6 mm is reported as particulate organic matter (POM).

Soil water fluxes of DON, NH_4^+, and NO_3^- were calculated from multiplying monthly concentrations from each lysimeter by the total soil water yield for that month. Soil water yield between lysimeter sample events was estimated using a V-notch weir located immediately uphill of the experimental plots, and which quantified water lost from a 1.7-ha forested watershed of comparable structure to our experimental area. Daily stream flow averaged 11 mm over the entire experimental period, with lowest flow during a period of Austral spring (September–October; 2.9 mm/d) and highest flow during a period in winter (June–July; 21.2 mm/d). Methods for watershed hydrologic balances are summarized in M. Walter, C. Salmon, M. Brown, and L. Hedin (*unpublished manuscript*). We did not adjust water yields by lysimeter depth; that is, we assumed an equal volume of water drained across a vertical plane at 10 cm vs. 40 cm soil depth. In reality, however, lysimeters at the two depths were most likely affected by factors such as evapotranspiration and preferential flow paths. We did not seek to quantify such effects.

Nitrogen content of most soil pools was calculated from estimates of N concentration and soil bulk density specific to each soil depth in each plot. The exception is coarse roots and POM, which are difficult to assess using small pits, and for which we used the N concentration measured in each plot multiplied by average biomass across all plots. We calculated ^{15}N recovery after one year based only on final N pools (Nadelhoffer et al. 1999) because C and N of samples collected prior to N additions did not vary systematically with assigned treatment level (Table 1). Litter samples collected at the end of the experiment were lost in transit, so ^{15}N recovery in litter could not be estimated. Tracer recoveries are expressed both as total recovery of ^{15}N, and as the distribution of recovered ^{15}N among component pools.

We examined whether ^{15}N recovery was nonlinear across the range of N inputs by testing for significant changes in ^{15}N recovery as a stepwise backwards function of N addition rate. We initiated the analysis using a linear regression of ^{15}N added vs. ^{15}N recovered for all treatment levels ($n = 9$), and asked whether the slope coefficient was sensitive to removal of the highest treatment level (i.e., test for homogeneity of slopes when comparing $n = 9$ vs. $n = 8$ treatments). We repeated this analysis by stepwise removal of treatment levels from highest to lowest, with testing for homogeneity of adjacent slopes (Sokal and Rohlf 1995). Tests were performed using GLM in SYSTAT 10.0 (SPSS, Chicago, Illinois, USA).

Gross NH_4^+ and NO_3^- cycling rates were calculated using standard equations (Hart et al. 1994). Gross heterotrophic NO_3^- production was determined by soil incubation in the presence of acetylene (Hart et al. 1997). Net NH_4^+ and NO_3^- production were estimated as the slope of inorganic N accumulation over 0, 10, and 28 d.

RESULTS

Leaching losses

On a concentration basis, dissolved organic nitrogen (DON) dominated N forms in soil water prior to N

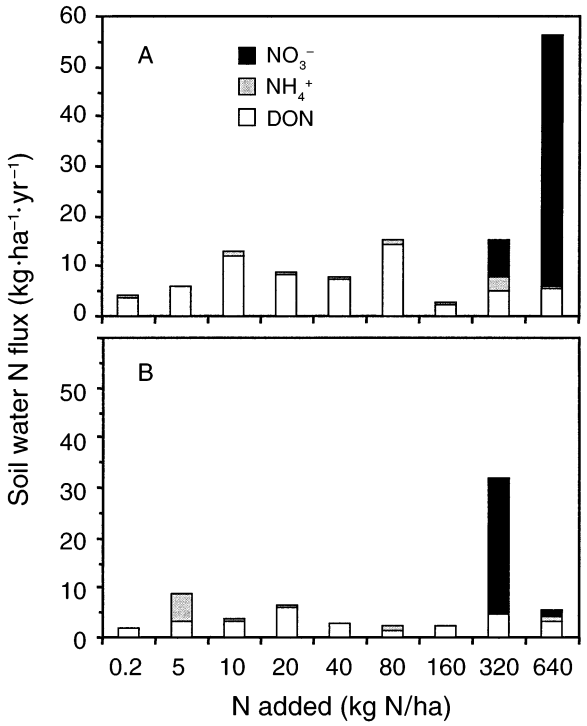

FIG. 1. Average annual soil water fluxes of NO_3^-, NH_4^+, and DON in lysimeters at (A) 10-cm and (B) 40-cm depth. Each value is the arithmetic mean based on 12 monthly samples.

additions (mean and standard error were 0.102 ± 0.04 mg N/L for seven shallow, and 0.070 ± 0.04 for seven deep lysimeters), with much lower concentrations of NH_4^+ (0.018 ± 0.02 mg N/L for seven shallow, and 0.024 ± 0.03 for seven deep lysimeters), and NO_3^- (0.008 ± 0.01 mg N/L for seven shallow, and 0.0006 ± 0.0001 for seven deep lysimeters). Three months after the start of N additions, we observed increases in soil water NO_3^- concentrations up to 6.8 mg N/L at the two highest N addition rates. Nitrate (NO_3^-) remained low in other treatments throughout 12 mo of N additions. Concentrations of NH_4^+ and DON were not significantly changed in any of the N addition treatments.

We show in Fig. 1 how these concentrations combined with estimated water flux translate into cumulative hydrologic N fluxes at 10-cm and 40-cm depth over the 12-mo N addition. Fluxes of N occurred mainly as DON at both depths for N addition rates ranging from ambient (0.2 kg·ha^{-1}·yr^{-1}) to 160 kg·ha^{-1}·yr^{-1}. However, at the two highest addition rates (320 and 640 kg N·ha^{-1}·yr^{-1}) we found dramatic increases in NO_3^- loss. While NO_3^- fluxes were most pronounced at 10-cm, they were still significant at 40 cm in both 320 and 640 kg N·ha^{-1}·yr^{-1} treatments; however, NO_3^- fluxes were not as high at 640 kg N·ha^{-1}·yr^{-1}, possibly owing to flow path effects. Overall, we found a maximum NO_3^- loss rate of 50.3 kg N·ha^{-1}·yr^{-1} for the 640 kg treatment at 10-cm depth, equivalent to ~8% of the total N added in this treatment.

Recovery of ^{15}N tracer

Total ^{15}N retention in 0–25 cm soils increased across the full range of N additions in our experiment (Fig. 2, Table 2). Tracer recovery averaged 52% across all treatments, with highest recovery (68%) in the 0.2 kg N/ha treatment, and lowest N recovery (44%) in the 320 kg N/ha treatment. Recovery of ^{15}N was a relatively constant proportion (slope = 62%) of added ^{15}N up to 160 kg N/ha treatment (dotted line in Fig. 2), and decreased at 320 and 640 kg N/ha addition rates (arrows in Fig. 2). Stepwise regression showed a statistically significant change in the slope of ^{15}N recovered vs. ^{15}N added between 160 and 320 kg N/ha treatments, indicative of nonlinear N retention efficiency at high rates of N input (Table 2). We note that our estimates of ^{15}N recovery at different rates of N addition are limited to one plot per treatment.

The distribution of recovered ^{15}N among soil depths and component pools revealed patterns and mechanisms of N retention as a function of N addition rate. Surface 0–10 cm soil accounted for the majority of recovered ^{15}N (average = 92%), regardless of addition rate. However, the mechanisms of ^{15}N retention in the surface 10 cm varied systematically as a function of N addition rate (Fig. 3). Soil organic matter accounted for over half of recovered ^{15}N at low additions, but retained a progressively smaller fraction as addition rate increased. In contrast, the distribution of ^{15}N in coarse roots and particulate organic matter (>5.6 mm) increased at higher addition rates. Microbial biomass and fine root pools retained constant proportions of ^{15}N across the range of addition rates. Extractable NH_4^+,

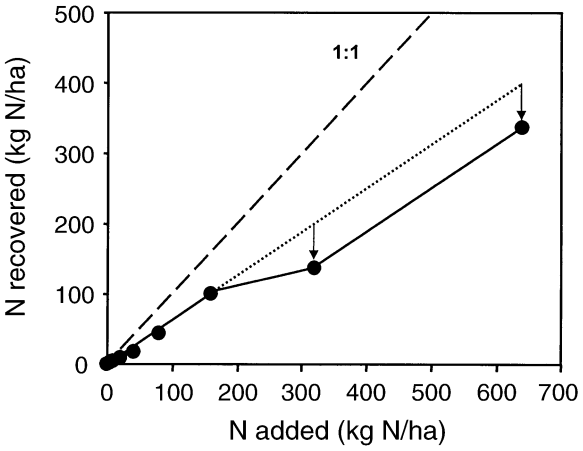

FIG. 2. N recovered vs. N added in 0–25 cm soil as determined by ^{15}N tracers. The solid line shows the measured pattern of N recovery. The dotted line shows expected the N recovery pattern based on linear regression that excludes the two highest treatments (see Table 2). Arrows show loss of N retention in treatments >160 kg N/ha. The dashed line shows the 1:1 recovery line.

TABLE 2. Results from least-squares regressions and tests of homogeneity of slopes to examine linearity in nitrogen recovery as a function of added nitrogen.

N added (kg/ha)	N recovered (kg/ha)	n	m	P_R	r^2	P_H
640	337.9	9	0.51	<0.01	0.98	0.23
320	136.7	8	0.46	<0.01	0.96	**0.03**
160	100.2	7	0.62	<0.01	0.99	0.09
80	44.2	6	0.55	<0.01	0.99	0.08
40	18.3	5	0.46	<0.01	0.99	0.68
20	9.3	4	0.47	0.01	0.99	0.38
10	5.3	3	0.52	0.05	0.99	0.58
5	2.3	2	0.45			
0.2	0.14	1				

Notes: Regressions of N recovered vs. N added were initiated using all treatment levels ($n = 9$), with recalculation following stepwise removal of the highest remaining value. Slope coefficients (m), the significance probabilities (P_R), and the coefficient of determination (r^2) are shown for each regression. P_H indicates the probability that slopes of adjacent n vs. $n - 1$ regression lines are significantly different. Boldface type signifies a significant change in the slope of N recovered vs. N added between 320 kg and 160 kg N/ha treatments. Nitrogen recovery was determined by ^{15}N tracers.

NO_3^-, and DON in soil each represented <1% of recovered ^{15}N (Appendix A). Despite variation in the relative importance (Fig. 3) and N pool sizes (Appendix B) of these different N retention mechanisms, all pools accumulated ^{15}N across the full range of N additions.

Soil nitrogen dynamics

We conducted detailed measurements of N pools and dynamics in 0–10 cm soils in order to assess the sensitivity of soil internal N cycling to added N. Experimental N additions increased N pool sizes of extractable NH_4^+ and NO_3^-, fine and coarse roots, and POM after one year, but did not significantly affect extractable DON, microbial biomass, or SOM (Appendix B). However, when microbial C and N are considered together, we found a significant logarithmic decline in microbial C:N with added N (Fig. 4). Standing biomass of fine and coarse roots, as well as SOM and POM, were not related statistically to N additions ($r^2 < 0.03$).

Net inorganic N production in field incubations was highest in 320 and 640 kg N/ha treatments (Table 3), but <5% was transformed to NO_3^-. Nitrate production was undetectable in potential nitrification of soil slurries amended with NH_4^+ (data not shown). The most probable number culture method found no detectable nitrifiers after three months incubation at 25°C (data not shown). Soil samples collected in 1997 prior to the start of N additions were incubated for 10 months, and we still found no nitrifiers.

Rates of gross inorganic N cycling did not vary systematically as a function of added N (Table 3). Gross NH_4^+ production averaged 13.2 mg N·kg soil^{-1}·d^{-1} (SE = 2.3) across all treatments. Nitrate accounted for 6% of gross inorganic N production, averaging 1.2 kg N·ha^{-1}·d^{-1} (SE = 0.5) across all treatments. Nitrification in the presence of acetylene averaged 82% of total nitrification (Table 3). Microbial respiration (soil CO_2 production in absence of roots) and K_2SO_4 extractable DOC did not vary systematically with added N either on an absolute basis, or per unit microbial biomass C and N (data not shown).

DISCUSSION

Nonlinear nitrogen retention

Our results support the idea (Aber et al. 1998) that the N cycle in forest ecosystems can change abruptly,

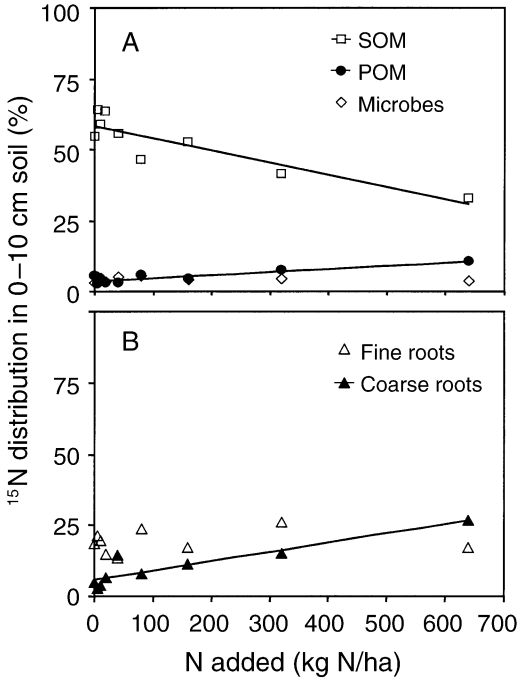

FIG. 3. Distribution of recovered ^{15}N in 0–10-cm soil depth as a function of N added for pools of (A) SOM (soil organic matter N <5.6 mm), POM (particulate organic matter N >5.6 mm), and microbial biomass N, and (B) live fine and coarse roots. Lines through symbols indicate significant linear regressions (all $r^2 > 0.80$, $P < 0.001$). Extractable NH_4^+, NO_3^-, and DON each accounted for <1% (Appendix A) and are not shown.

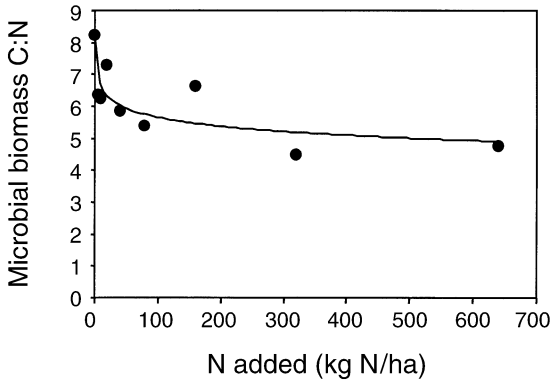

FIG. 4. Microbial C:N (by mass) in 0–10 cm soil as a function of N added. Equation of line: $y = -0.40 \ln(x) + 7.51$; $r^2 = 0.70$, $P < 0.01$.

in a nonlinear fashion, in response to elevated inputs of N. In our study, this nonlinearity appeared as a threshold of reduced ^{15}N retention, increased soil net inorganic N production, and accelerated leaching of N as NO_3^- where N additions exceeded 160 kg $N \cdot ha^{-1} \cdot yr^{-1}$ (Figs. 1 and 2). This rate of N addition is 5–10-fold greater than chronic N pollution in many temperate forests, yet is comparable to N inputs from pure stands of symbiotic N fixers and forest fertilization (Johnson 1992, Cleveland et al. 1999). We note that even though N retention efficiencies in our plots were roughly comparable to the range observed in other studies, our N additions extended only over 12 months, and therefore did not evaluate the longer term impacts of particular addition rates on soil N cycling and loss. Nevertheless, our results suggest that N balances of unpolluted old-growth temperate forests may be sensitive even over very short time scales to both natural and anthropogenic increases in N supply.

The rapid transition from N retentive to N leaky that occurred in our experiment was more complicated than suggested from simple dose–response relationships. First, NO_3^- loss remained consistently low in plots that received 80 and 160 kg N/ha over 12 months, even though these same cumulative amounts of N input increased soil water NO_3^- sharply after only three months in the two highest treatments (320 and 640 kg $N \cdot ha^{-1} \cdot yr^{-1}$). Second, the abrupt increase in potential NO_3^- flux in soil water (8–50 kg $N \cdot ha^{-1} \cdot yr^{-1}$) remained low relative to the capacity of soils to retain ^{15}N (137–338 kg $N \cdot ha^{-1} \cdot yr^{-1}$) in the two highest treatments. Third, every soil component pool that we measured (i.e., organic matter, plant roots, microbial biomass) accumulated progressively greater quantities of ^{15}N tracers on an absolute basis (kg N/ha) across the full range of N additions (Appendix A). Together these results highlight how accelerated N inputs can promote transient N leakiness and N accumulation simultaneously in terrestrial ecosystems. This resembles how most forest ecosystems process accelerated N inputs even after years to decades of elevated N supply (Gunderson et al. 1998). To explain this pattern, progressively larger N additions must stimulate faster yet sloppier N uptake, resulting from increased rates of N incorporation and declines in overall N retention efficiency. Consequently, the capacity for ecosystem N uptake may not be a static system property, but instead may be malleable as a function of N supply. We conclude that the rate (i.e., kinetics) of N uptake may be as important as, and possibly influence, the capacity (i.e., integrated amount) for N uptake as controls of ecosystem N input–output balances.

The theory of N saturation developed by Ågren and Bosatta (1988) predicts that changes in microbial C:N ratios are the most important control of N saturation in the plant–soil–microbe subsystem. Specifically, a decrease in microbial C:N is thought to lower the critical C:N ratio for net N mineralization from the large slowly cycling pool of SOM, thus increasing the N storage capacity of SOM. According to this mechanism alone, with no other changes in controls of SOM stoichiometry (i.e., litter quality, microbial efficiency, C:N of hydrologic losses), we adopt the approach of Ågren and Bosatta (1988) to calculate that the decline in microbial C:N from 8.4 to 4.8 observed across our N gradient (Fig. 4) could result in a proportional 40% increase in N storage in SOM by lowering the critical

TABLE 3. Gross and net nitrogen production following one year of nitrogen additions.

N added (kg/ha)	Net N production			Gross N production			
	NH_4^+	NO_3^-	NO_3^- (%)	NH_4^+	NO_3^-	NO_3^- (%)	Heterotrophic (%)
0.2	0.09	0.002	2	9.7	0.8	8	100
5	0.00	0.000	NA	14.6	0.2	1	67
10	0.20	<0.001	0	9.9	0.4	4	92
20	0.10	0.004	4	14.6	0.0	0	NA
40	0.40	0.003	1	26.2	4.0	13	64
80	0.00	0.000	NA	17.3	1.4	8	70
160	0.00	0.000	NA	6.9	0.8	10	83
320	1.08	<0.001	0	2.3	0.0	0	NA
640	2.53	<0.001	0	17.4	2.8	14	100

Notes: Gross NO_3^- production includes measured contributions from autotrophic and heterotrophic pathways. Percentages of NO_3^- measured in net and gross rates are corrected to prevent double counting of nitrate produced by ammonium oxidation. Rates are expressed as $\mu g \ N \cdot (g \ soil)^{-1} \cdot d^{-1}$. NA indicates percentages not calculated due to nondetectable NO_3^- production.

C:N for net N mineralization. This long-term N sink is quantitatively significant at the ecosystem level, since SOM accounts for 80% of total N in this forest (Vann et al. 2002). In contrast, the direct storage potential for N in microbial biomass is small at all rates of N addition (Fig. 3B), since this pool turns over rapidly, and accounts for only several percent of total N and ^{15}N. A decline in microbial C:N may be more important than changes in microbial biomass N pool size when considering how long-term N additions will shape the N retention capacity of forest ecosystems.

Shifts in nitrogen cycling pathways

The relative shifts in ^{15}N distribution among plant–soil–microbial component pools reveal how pathways of N retention vary as functions of N supply (Fig. 3). Soil and particulate organic matter (POM) combined to account for 43–67% of total recovered ^{15}N, highlighting the importance of detritus as a N retention mechanism in forest ecosystems (Currie et al. 1999). Few tracer studies assess ^{15}N recovery in larger size classes of organic matter (e.g., Schimel and Firestone 1989). Yet we were surprised to find that our operational size distinction between soil and particulate fractions (5.6 mm cutoff) corresponded to divergent patterns of ^{15}N distribution in organic matter pools. The relative importance of SOM (<5.6 mm) as a ^{15}N sink decreased steadily across the range of N additions, whereas ^{15}N distribution in larger POM size fractions increased (Fig. 3). When added together, the efficiency of N retention in these organic matter pools decreased significantly across our N gradient ($r^2 = 0.76$, $P = 0.002$, $n = 9$), suggesting that microbial transfer of N to POM does not offset the loss of N immobilization efficiency in SOM. Nevertheless, high biomass of coarse woody organic matter is a characteristic feature of many old-growth temperate ecosystems (Spies et al. 1988, Carmona et al. 2002). Consequently, short-term net N immobilization in high C:N woody detritus may explain why some old-growth forests retain N inputs more efficiently than second-growth forests (Fisk et al. 2002).

Plant–microbial competition for N is considered a dominant control on terrestrial N cycling, and it is widely accepted that high N inputs can enhance plant access to N by saturating microbial demands (Johnson 1992, Kaye and Hart 1997, Magill et al. 2000). We interpret the increased ^{15}N recovery in coarse roots to indicate that plants became better competitors for N with increased N additions (Miller et al. 1976, Nadelhoffer et al. 1999). It is likely that a large fraction of unrecovered ^{15}N was transported through roots to unmeasured aboveground sinks in vegetation, since our sampling methods have been shown to recover 100% of soil ^{15}N tracers (Perakis and Hedin 2001), and since soil water NO_3^- fluxes were a small proportion (average = 1%, range = 0–8%, $n = 9$) of applied N across all treatments. The constant distribution of ^{15}N in fine roots, coincident with increased coarse root ^{15}N, may be explained by accelerated turnover and/or redistribution of N from fine to coarse roots with increasing N supply. At our highest N addition rate, coarse roots accounted for an unexpected 27% of recovered ^{15}N (14% of total applied ^{15}N). These results suggest that coarse roots and POM are important yet overlooked sinks for N in forest ecosystems, and may account for a substantial portion of the ''missing N'' often reported in ^{15}N tracer and chronic N studies.

Perhaps our most surprising result was a failure of N additions to stimulate autotrophic NO_3^- production. Aber et al. (1998) hypothesized that added N would increase NH_4^+ availability and stimulate autotrophic nitrifying bacteria, thus promoting N losses via increased NO_3^- leaching. Soil NH_4^+ did increase in our study (Appendix B), as did NO_3^- leaching (Fig. 1). Yet heterotrophic pathways dominated NO_3^- production across the full range of N additions, with no change in gross autotrophic NO_3^- production, potential autotrophic nitrification, or numbers of nitrifying bacteria (Table 3). Other studies also have found limited potential for nitrification following large N additions (Christ et al. 1995), confirming the idea that ammonium oxidation may be of limited importance to internal N cycling in some acid forest soils even when NH_4^+ is abundant (Schimel et al. 1984, Hart et al. 1997).

It is unclear why our N additions failed to stimulate autotrophic nitrification. Although optimal abiotic conditions for nitrification are site and soil dependent, soils in our study were sufficiently moist (average, 3 g H_2O/g soil; range, 1.2–6.5; $n = 9$), warm (12°C), and oxygenated (W. L. Silver, *unpublished data*) at the time of sampling that these factors are unlikely to have prevented autotrophic nitrification. End-product inhibition by added NO_3^- is also unlikely, since our most probable numbers assay did not detect the bacteria that regulate NH_4^+ oxidation to NO_2^-, which should not be affected by NO_3^- accumulation. We cannot rule out the possibility that strong competition for NH_4^+, acidic conditions ($pH_{water} = 4.6$; Perez et al. 1991), allelopathy (Rice and Pancholy 1972), or some other factors have selected against ammonium oxidizer populations or activities in these forests. Stimulation of autotrophic nitrifiers may require longer term increases in N supply than occurred in our study, as suggested by DNA and enzyme profiles of soil microorganisms in control vs. N-treated plots of the Harvard Forest chronic N experiments (Compton et al. 2004). Regardless of the cause, autotrophic nitrification appears to play a relatively minor role in the N cycle of this forest.

Incomplete assimilation of NO_3^-, rather than accelerated nitrification, thus appears to explain the elevated NO_3^- concentrations we observed in field net N incubations and soil water leachates. Major mechanisms of NO_3^- assimilation in temperate forest soils include uptake by plants, mycorrhizae, free-living heterotrophs, and abiotic reactions with organic matter. Plant

uptake is unlikely to have decreased at high N supply, since ^{15}N recovery in coarse roots increased across the gradient of N additions (Fig. 3B). Short-term N processing by free-living heterotrophs is unlikely to have increased substantially with added N, since negligible responses were observed in gross N cycling, soil extractable C, microbial C, and soil CO_2 respiration. Moreover, microbial C:N declined sharply at low rates of N addition relative to inputs that stimulated excess NO_3^- loss. However, NO_3^- incorporation by mycorrhizae (Aber et al. 1998) and/or rapid abiotic reactions (Perakis and Hedin 2001, Davidson et al. 2003) could have declined in response to added N without coincident changes in gross N cycling, extractable and microbial C, or soil respiration. Saturation of mycorrhizae and abiotic reactions may explain the decreased efficiency of ^{15}N incorporation into SOM across our N supply gradient, and provides mechanisms for accelerated net NO_3^- production and loss when autotrophic nitrification is negligible.

Fluxes of soil water NO_3^- increased dramatically in 320 and 640 kg $N \cdot ha^{-1} \cdot yr^{-1}$ treatments, from low background levels (0.001 kg $N \cdot ha^{-1} \cdot yr^{-1}$, $<1\%$ of dissolved N fluxes) characteristic of unpolluted forests to values more typical of forests receiving heavy N loads (8–50 kg $N \cdot ha^{-1} \cdot yr^{-1}$, 50–90% of dissolved N fluxes). Soil water DON fluxes were unrelated to N inputs, reinforcing the idea that these solutes are linked only indirectly to actively cycling soil pools (Hedin et al. 1995, Perakis and Hedin 2001). Long-term (decade to century) increases in N supply appear necessary to enhance losses of DON to any measurable degree (Currie et al. 1996, Compton et al. 2003), yet such chronic N inputs are even more apt to bias N losses in favor of NO_3^-. Consequently, theories that consider DON loss as a key driver of forest nutrient limitation (Hedin et al. 1995, 2003, Vitousek et al. 1998) and response to climatic change (Rastetter et al., *in press*) may require modification to encompass the changes in N cycling and loss that arise following N inputs that are typical of forest fertilization, symbiotic fixation, and/or chronic atmospheric pollution.

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APPENDIX A

A table showing recovery of ^{15}N additions after one year is available in ESA's Electronic Data Archive: *Ecological Archives* E086-006-A1.

APPENDIX B

A table showing nitrogen pools after one year of N additions is available in ESA's Electronic Data Archive: *Ecological Archives* E086-006-A2.